



Anti-leishmanial activity of neolignans from *Viola* species and synthetic analogues[☆]

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Abstract

Surinamensin, a neolignan isolated from *Viola surinamensis*, 3,4,5-trimethoxy-8-[2',6'-dimethoxy-4'-(E)-propenylphenoxy]-phenylpropane, a neolignan isolated from *Viola pavonis*, and 25 of its synthetic analogues or correlated substances with ether linkages and their corresponding C-8 sulphur and nitrogen analogues, were tested for activity against *Leishmania donovani* amastigotes and promastigotes in vitro. Some were active against *L. donovani* promastigotes at 30 μ M but inactive against intracellular amastigotes. The natural neolignan from *V. pavonis* was active against promastigotes at 100 μ M. The highest selective activity was found in those compounds with sulphur bridges. The β -ketosulfide (3,4-dimethoxy)-8-(4'-methylthiophenoxy)-propiophenone produced 42% inhibition of *L. donovani* amastigotes in the liver of BALB/c mice at 100 mg/kg given once daily for five consecutive days ($P > 0.05$). © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Viola surinamensis*; *Viola pavonis*; Myristicaceae; *Leishmania donovani*; β -Ketoethers; β -Ketosulfides; β -Ketoamines; Neolignans; Anti-leishmanial activity

1. Introduction

Leishmaniasis is a severe debilitating disease, infecting an estimated 1,400,000 people annually worldwide (Ashford et al., 1992), many of whom live in rural areas without access to treatment. The disease is caused by haemoflagellate protozoan parasites that survive and multiply in macrophages in the mammalian host and are transmitted by phlebotomine sandflies. The first choice of drugs, the pentavalent antimonials, sodium stibogluconate and meglumine antimoniate, require parenteral administration under medical supervision, and side effects frequently occur (Olliaro and Bryceson, 1993; Berman, 1997). Although a few new drugs, such as the lipid amphotericin B formulations, paromomycin, ketoconazole

and allopurinol are currently on clinical trial, results for some have been equivocal while others still depend upon parenteral administration. There remains an urgent need for new therapeutic agents (Olliaro and Bryceson, 1993; Berman, 1997). In studies to identify new anti-leishmanial compounds, a range of plant products, including indole and isoquinoline alkaloids, chalcones, quinones and terpenes, showed activity against *Leishmania* spp. (Wright and Phillipson, 1990; Iwu et al., 1994); licochalcone A and propylquinolone derivatives look particularly promising (Chen et al., 1994; Fournet et al., 1996). Diarylheptanoids, an iso-flavonoid (Araujo et al., 1998) and a chalcone were also active in *L. amazonensis* (Torres-Santos et al., 1999a,b). Advances in the fight against leishmaniasis using natural products have been recently reviewed (Akendengue et al., 1999).

As part of a programme to identify novel anti-leishmanial compounds, natural and synthetic neolignans, derivatives and analogues, were tested for activity against *L. donovani* in culture and animal models. Neolignans,

[☆] In memoriam of Ralph A. Neal^c.

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which occur in the Myristicaceae and other primitive plant families, are usually dimers of the oxidative coupling of allyl and propenyl phenols (Gottlieb, 1978). They have a wide range of biological effects including antifungal, anti-inflammatory, neuroleptic, antihepatotoxic, anticancer and anti-PAF activities (MacRae and Towers, 1984; Lima et al., 1987; Barata et al., 1991; Santos, 1991). QSAR studies of synthetic neolignans and related active compounds against *L. donovani* have recently been correlated (Costa et al., 1995, 1999).

2. Results and discussion

The natural neolignans used in this study were isolated from *Virola surinamensis* (Rol.) Warb and *V. pavanis* (A.DC.) A.C. Smith leaves, (Myristicaceae), collected in the Amazon rainforest, Brazil. Surinamensin **1a** and virolin **1b**, biologically active 8-*O*-4' neolignans (Gottlieb, 1978; Oliveira and Sampaio, 1978), were previously isolated from *V. surinamensis* (Barata et al., 1978). The absolute configuration of **1a** was determined using diastereomeric *O*-methyl mandelic esters (Santos and Barata, 1990). The 8-*O*-4'-neolignan 3,4,5-trimethoxy-8-[3',5'-dimethoxy-4'-(*E*)-propenylphenoxy]-phenylpropane (**20**), was isolated from *V. pavanis* as previously described (Ferri and Barata, 1992).

β -Ketoether compounds (**2–18**) were obtained as previously described (Santos and Barata, 1990; Barata et al., 1991; Santos, 1991). 2-methyl-3-(4'-hydroxy-3'-methoxyphenyl)-1,4-(2''-hydroxy)-benzodioxane (**19**) and 4-hydroxy-3-methoxy-8-[3',5'-dimethoxy-4'-(*E*)-propenylphenoxy]-phenylpropane (**21**) were synthesized as reported (Ferri and Barata, 1992). The β -ketoamines 8-anilinium-propiofenone (**22**) and (3,4-dimethoxy)-8-

anilinium-propiofenone (**23**), and the β -ketosulfides 8-(4-chlorophenoxy)-propiofenone (**24**), 3,4-dimethoxy-8-(4'-methylthiophenoxy)-propiofenone (**25**), 3,4-dimethoxy-8-(4'-chlorothiophenoxy)-propiofenone (**26**) and 3,4-dimethoxy-8-(thiophenoxy)-propiofenone (**27**), were synthesized through Friedel-Crafts reaction of a benzene derivative, bromination of the resultant aromatic ketone with bromine, condensation in basic medium of the appropriate α -bromoketone with aniline or a derivative and with thiophenol or a derivative. These furnished the β -ketoamines and the β -ketosulfides (respectively) in good yield. Alternatively, the α -bromoketone could be generated by hydro-bromination of abundant Brazilian natural products such as *iso*-eugenol or *iso*-safrole with NBS:H₂O leading to a bromidrine with posterior oxidation (Santos et al., 1992). All spectral data of synthesized compounds were in agreement with the expected products. The structure of β -keto-sulfide **27** was confirmed through X-ray analysis (Larucci et al., 1995).

The activity of compounds **1a** and **2–27** was assessed against *L. donovani* promastigotes and amastigotes in vitro, (Tables 1 and 2), the activity of 8-phenoxy-acetophenone (**7**) and compounds **24–27** was assessed in vivo.

Surinamensin **1a**, a neolignan isolated from *Virola surinamensis* and selected for its anti-schistosomal activity (Barata et al., 1978), was active in vitro against *L. donovani* promastigotes at 50 μ M, but showed no selective toxicity when tested against *L. donovani* amastigotes in the mouse peritoneal macrophage model. In an assay using extracellular promastigotes, 3,4-dimethoxy-8-[3'-methoxy-4'-(*E*)-propenylphenoxy]-propiofenone (**2**), 3,4-methylenedioxy-8-[3'-methoxy-4'-(*E*)-propenylphenoxy]-propiofenone (**3**), 3,4-dimethoxy-8-(4'-methoxyphenoxy)-propiofenone (**12**), 3,4-dimethoxy-8-[3'-methoxy-4'-(*E*)-allylphenoxy]-propiofenone (**16**), and compounds **19**, **21** and **24–26** were active in the range of 30 and 100 μ M (Table 1). The natural neolignan **20** from *V. pavanis* was active at 100 μ M. The rest of the compounds were inactive.

In tests against amastigotes in macrophages, **1a**, **2**, 7-hydroxy-8-phenoxy-phenylethane (**4**), 8-phenoxy-acetophenone-oxime (**6**), 3,4-dimethoxy-8-(4'-chlorophenoxy)-propiofenone (**15**), 8-(3'-methylphenoxy)-acetophenone (**18**), **19**, **22** and **23** were active at 100 μ M but also toxic to macrophages, while 3,4-dimethoxy-8-(2'-methoxyphenoxy)-propiofenone (**14**) and **15** were selectively toxic to macrophages. **3**, 7-Amine-8-phenoxy-phenylethane (**5**), **16**, 3,4-dimethoxy-8-[2'-(1',3'-dimethoxyphenoxy)]-propiofenone (**17**) and **27** were active at 100 μ M against intracellular amastigotes, while 8-(2'-nitrophenoxy)-acetophenone (**11**) showed significant activity against intracellular amastigotes at 30 μ M (Table 2) although it was not active against promastigotes. Compounds **24**, **25** and **26**, (all with a sulphur bridge) were active against both amastigotes and promastigotes

Table 1

In vitro anti-*L. donovani* promastigote activity of synthetic neolignan analogues

Compound ^{a,b}	Inhibition at 100 μ M ^c	Inhibition at 30 μ M
2	++++	++++
3	++++	+++
12	++++	+++
16	++	–
19	+++	++
21	++	–
24	++	–
25	+++	++
26	++++	+++

^a Compounds **4–11**, **13–15**, **17**, **18**, **22**, **23** and **27** were inactive at 100 μ M.

^b Compound **20**, a natural neolignan from *V. pavanis*, produced +++ inhibition at 100 μ M.

^c Key: ++++ All promastigotes rounded up and non-motile after 48 h; +++ all promastigotes rounded up and only slight movement visible after 48 h; ++ Moderate inhibition of promastigote motility after 48 h; + Slight inhibition of promastigote motility after 48 h.

Table 2
In vitro anti-*L. donovani* amastigote activity of synthetic neolignan analogues

Compound	%I ^a at 100 µM ± S.E.	%I at 30 µM ± S.E.	%I at 10 µM ± S.E.	ED ₅₀	Toxic to MØs at
3	90.7±0.5	10.6±1.4	0±0	50.05 (54.26–46.39)	100 µM
5	90.2±0.5	0±0	0±0		300 µM
11	100±0	91.9±0.6	0±0		100 µM
16	91.9±0.3	0±0	0±0		300 µM
17	41±6.7	0±0	0±0		300 µM
24	100±0	46.3±5	0±0	27.69 (29.66–25.52)	Not toxic at 300 µM
25	100±0	70.6±3.3	0±0	24.32 (26.52–21.73)	Not toxic at 300 µM
26	100±0	90.2±0.9	0±0	IC ₅₀	Not toxic at 300 µM
27	83.3±1.5	0±0	0±0		Not toxic at 300 µM
	%I at 27 µg Sb ^v /ml±S.E.	%I at 9 µg Sb ^v /ml±S.E.	%I at 3 µg Sb ^v /ml±S.E.		
Na-stibogluconate	81.3±1.5	77.4±5.2	23.0 ±3.8	5.32 (5.8197–4.8182)	

^a % I = % Inhibition of amastigotes.

(Tables 1 and 2) and showed the greatest selectivity. 8-(2'-methoxyphenoxy)-acetophenone (**8**), 8-(4'-methoxyphenoxy)-acetophenone (**9**), 8-(4'-chlorophenoxy)-acetophenone (**10**) and 3,4-dimethoxy-8-phenoxy-propiofenone (**13**) were inactive at 100 µM against promastigotes and amastigotes. Some of the compounds which showed *in vitro* activity were further tested against *L. donovani* in the BALB/c mouse model. Only compound **25** showed significant anti-leishmanial activity *in vivo*, reducing the liver amastigote load by 42% ($P > 0.05$) at a dose of 100 mg/kg/day for 5 days (Table 3).

3. Conclusions

β-Ketoethers **3**, **5**, **11**, **16** and **17** and β-ketosulfides **24**, **25**, **26** and **27** were selectively toxic to amastigotes *in vitro*, but **24**, **25** and **26** showed the greatest selectivity, suggesting that the C8 sulphur bond may play a part in the anti-*L. donovani* activity of these compounds. However, only compound **25** was significantly active *in vivo*, producing the greatest reduction in amastigote liver

load. Compounds **5**, **11** and **17** were not active against promastigotes, although they were active against amastigotes. This may be due to biochemical or metabolic differences between the two stages of the parasite, or to variations in the intracellular concentration of the drug by the extracellular promastigotes and the macrophages.

Preliminary mode of action studies on promastigotes of β-ketosulphides compounds showed that they induce aberrant non-motile forms, with displaced nuclei and absent or reduced flagella, suggesting microtubule inhibition. Further studies are needed in order to elucidate the mechanism(s) of action of these compounds.

4. Experimental

4.1. General experimental procedures

Mps. (uncorr.) were taken on a Köfler microscopic apparatus. NMR spectra were determined on a Bruker AW-80 instruments (80 MHz) and a 300 MHz in CDCl₃ solution, using TMS as internal reference. Chemical shifts are expressed as δ values and coupling constants (J) are expressed in Hertz (Hz). IR spectra were recorded in KBr film and measured with a Perkin-Elmer model 399B spectrophotometer. EIMS were obtained by direct probe insertion at 70 eV.

4.2. Plant material

Leaves of *Virola pavanis* (A.DC) A.C. Smith (Myristicaceae) were collected in the Amazon forest by Dr. Hipólito F. Paulino Filho, near the Guaporé river, State of Rondônia, Brazil, and authenticated by Dr. William A. Rodrigues (Instituto Nacional de Pesquisa da Amazônia). A voucher specimen is deposited in the herbarium of INPA, Manaus, Brazil. Leaves of *Virola surinamensis* (Rol.) Warb were collected in Belém, State of Pará, Brazil. A voucher specimen (N° 166061) has

Table 3
In vivo activity of synthetic neolignan analogues and sodium stibogluconate against *L. donovani* in BALB/c mice^a

Compound	Dose level (mg/kg/day for 5 days)	%I
7	100	26
24	100	–1
25	100	42
		$P > 0.05$
26	100	–19
27	100	17
Na-stibogluconate	45 (mg Sb ^v /kg/day)	97
		$P > 0.05$
	15 (mg Sb ^v /kg/day)	74
		$P > 0.05$

^a $P > 0.05$ indicates the significant difference between number of parasites in the drug treated mice compared with the untreated controls.

been deposited in the herbarium of Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA-Divisão de Botânica), Belém-PA, Brazil.

4.3. Isolation

Surinamensin **1a** was isolated from the leaves of *V. surinamensis* as previously described (Barata et al., 1978; Santos and Barata, 1990). 3,4,5-Trimethoxy-8-[3',5'-dimethoxy-4'-(E)-propenylphenoxy]-phenylpropane (**20**) was isolated from *V. pavonis* as previously described (Ferri and Barata, 1992).

4.4. General procedure for synthesis of β -ketoamines

A solution of the α -bromoketone (1 equiv.) (Barata et al., 1978; Santos and Barata, 1990; Santos, 1991) in treated butanone (5 ml of solvent/mmol of ketone) was added to a mixture of the aromatic amine (2.5 equiv.) in butanone (5 ml of solvent/mmol of amine) and NaHCO_3 (1.1 equiv.). The reaction was stirred at 80°C for 3–6 h. It was then left to cool, and H_2O was added and the solution was extracted with CH_2Cl_2 . The organic phase was washed with 2% HCl solution, dried (Na_2SO_4), filtered and evaporated at reduced pressure. The crude residue was recrystallized from MeOH to produce an 80–82% yield of the following β -ketoamines.

4.4.1. 8-Anilinium-propiofenone **22**

The crude product obtained from 1.50 g (16.13 mmol) of aniline, 0.60 g (7.11 mmol) of NaHCO_3 and 1.37 g (6.46 mmol) of 3,4-dimethoxy-8-bromo-propiofenone after 3 h of reaction was recrystallized from MeOH affording 1.90 g of **22** as crystalline solid, mp 103.0–105.0°C (82% yield). ^1H NMR spectral data (80 MHz, CCl_4): δ 1.50 (3H, *d*, $J=7.0$ Hz, H-9), 4.50–4.80 (1 H, *m*, NH), 5.10 (1H, *q*, $J=7.7$ Hz, H-8), 6.20–8.20 (8H, *m*, H-Ar).

4.4.2. (3,4-Dimethoxy)-8-anilinium-propiofenone **23**

The crude product obtained from 1.70 g (18.31 mmol) of aniline, 0.68 g (8.10 mmol) of NaHCO_3 and 2.00 g (7.30 mmol) of 3,4-dimethoxy-8-bromo-propiofenone after 6 h of reaction was recrystallized from MeOH producing 1.68 g of **23** as a crystalline solid, mp 109–110°C (80% yield). $\text{IR } \nu_{\text{max}}^{\text{KBr}}$ (cm^{-1}): 3370 (NH), 1670 (C=O); ^1H NMR spectral data (300 MHz, CDCl_3 : CCl_4 , 8:2): δ 1.45 (3H, *d*, $J=8.8$ Hz, H-9), 3.89 (3H, *s*, OMe-3), 3.84 (3H, *s*, OMe-4), 4.48 (1H, *m*, NH), 5.15 (1H, *q*, $J=8.8$ Hz, H-8) 6.50–7.70 (8H, *m*, H-Ar).

4.5. General procedure for synthesis of β -ketosulfides

A solution of 1.03 equiv. of thiophenol or derivative and 1.8 g of dry K_2CO_3 in treated ethyl methyl ketone

(4.5 ml of solvent/mmol of thiophenol) was stirred for 10 min at room temp. After this period, a solution of α -bromoketone in ethyl methyl ketone (1.5 ml of solvent/mmol of ketone) was added dropwise and the reaction mixture was treated until reflux began, this being maintained for 6–8 h. The cooled solution was filtered and the residue washed with CH_2Cl_2 . The resultant filtrate was conc., diluted with H_2O and extracted thoroughly with CH_2Cl_2 ($\times 4$). Organic extracts were combined, washed with 5% NaOH solution, satd. NaCl soln. and dried (Na_2SO_4). The solution was filtered, concentrated under reduced pressure, to give after purification, 78–85% yields of the β -ketosulfides.

4.5.1. 2-(4-Chlorophenoxy)-propiofenone **24**

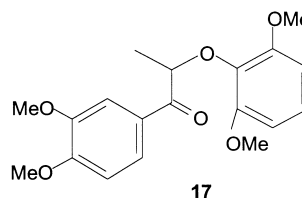
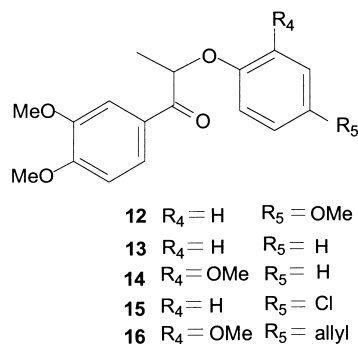
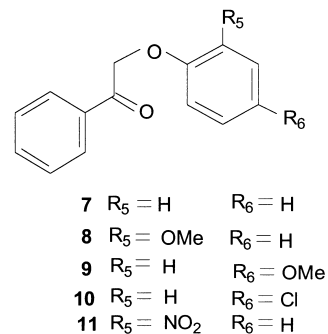
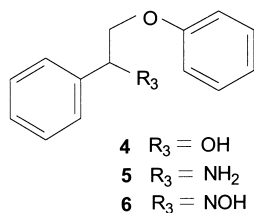
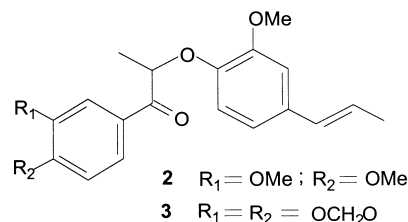
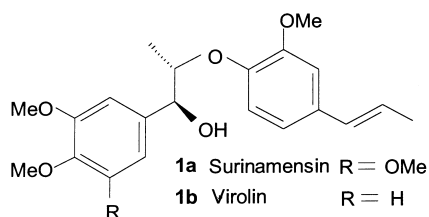
The crude residue obtained from 4-chlorothiophenol (1.40 g, 9.67 mmol) of K_2CO_3 (2.33 g, 16.99 mmol) and of 8-bromo-propiofenone (2.0 g, 9.39 mmol) after 7 h of reaction was recrystallized from MeOH yield **24** (2.02 g) as colorless crystals, mp 70.0–71.5°C (78% yield). $\text{IR } \nu_{\text{max}}^{\text{KBr}}$ (cm^{-1}): 3060, 2820, 1675 (C=O), 1595, 1580; ^1H NMR spectral data (80 MHz, CCl_4): 1.44 (3H, *d*, $J=7.0$ Hz, H-9), 4.46 (1H, *q*, $J=7.0$ Hz, H-8), 7.10–8.05 (9H, *m*, H-Ar); EIMS (probe) 70 eV, *m/z* (rel. int.): 276 $[\text{M}]^+$ (34), 171 $[\text{C}_8\text{H}_8\text{SCl}]^+$ (52), 143 $[\text{C}_6\text{H}_5\text{SCl}]^+$ (11), 105 $[\text{C}_7\text{H}_5\text{O}]^+$ (100).

4.5.2. (3,4-Dimethoxy)-8-(4'-methylthiophenoxy)-propiofenone **25**

The crude residue obtained from 4-methylthiophenol, (1.87 g, 15.08 mmol) K_2CO_3 (3.64 g, 26.37 mmol) and 3,4-dimethoxy-8-bromo-propiofenone (4.62 g, 14.62 mmol) after 7.0 h of reaction, was recrystallized from MeOH to give of **25** (3.87g) as colourless crystals, mp 80.0–81.0°C. $\text{IR } \nu_{\text{max}}^{\text{KBr}}$ (cm^{-1}): 3040, 2840, 1665 (C=O), 1580, 1570, 1380; ^1H NMR spectral data (80 MHz, CDCl_3): δ 1.40 (3H, *d*, $J=7.0$ Hz, H-9), 2.30 (3H, *s*, H-1'), 4.36 (1 H, *q*, $J=7.0$ Hz, H-8), 6.60–7.60 (6H, *m*, H-Ar); EIMS (probe) 70 eV, *m/z* (rel. int.): 316 $[\text{M}]^+$ (15), 165 $[\text{C}_9\text{H}_9\text{O}_3]^+$ (100), 151 $[\text{C}_9\text{H}_{11}\text{S}]^+$ (12), 123 $[\text{C}_7\text{H}_7\text{S}]^+$ (7).

4.5.3. (3,4-Dimethoxy)-8-(4'-chlorothiophenoxy)-propiofenone **26**

The crude residue obtained from 4-chlorothiophenol, (1.36 g, 9.43 mmol) K_2CO_3 (2.27 g, 16.48 mmol) and 3,4-dimethoxy-8-bromo-propiofenone (2.50 g, 9.16 mmol) after 8.0 h of reaction, was recrystallized from MeOH to give **26** (2.52 g) as colorless crystals, mp 90–94°C (81.7% yield). $\text{IR } \nu_{\text{max}}^{\text{KBr}}$ (cm^{-1}): 3085, 2850, 1670 (C=O), 1595, 1585, 1480. ^1H NMR spectral data (80 MHz, CDCl_3): δ 1.50 (3H, *d*, $J=7.0$ Hz, H-9), 3.86 (3H, *s*, OMe-3) 3.92 (3H, *s*, OMe-4), 4.59 (1H, *q*, $J=7.0$ Hz, H-8), 6.70–7.80 (7H, *m*, H-Ar); EIMS (probe) 70 eV, *m/z* (rel. int.): 336 $[\text{M}]^+$ (10), 165 $[\text{C}_9\text{H}_9\text{O}_3]^+$ (100).



4.5.4. (3, 4-Dimethoxy)-8-(thiophenoxy)-propiophenone **27**

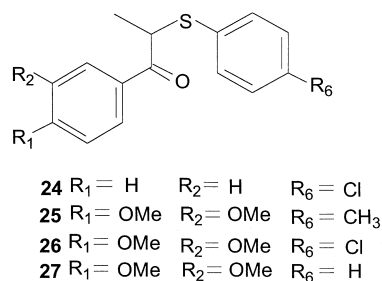
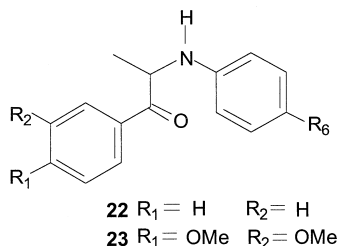
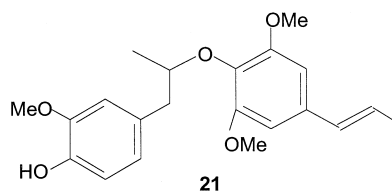
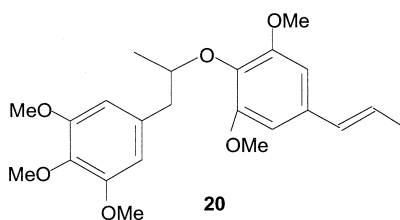
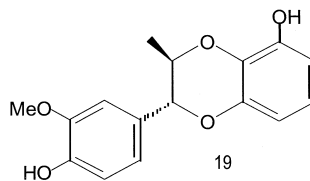
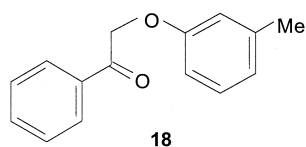
The crude residue obtained from thiophenol, (2.07 g, 18.86 mmol) K₂CO₃ (4.55 g, 32.97 mmol) and 3,4-dimethoxy-8-bromo-propiophenone (5.0 g, 18.31 mmol) after 6.0 h of reaction was recrystallized from MeOH to give **27** (4.70 g) as colorless crystals, mp 59.0–61.0°C (85% yield). IR_ν^{KBr} (cm⁻¹): 3060, 2840, 1670 (C=O), 1590, 1580, 1520, 1470, 1275; ¹H NMR spectral data (80 MHz, CDCl₃): δ 1.52 (3H, *d*, *J* = 7.0 Hz, H-9), 3.88 (3H, *s*, OMe-3), 3.92 (3H, *s*, OMe-4), 4.60 (1 H, *q*, *J* = 7.0 Hz, H-8), 6.70–7.80 (8H, *m*, H-Ar); EIMS (probe) 70 eV, *m/z* (rel. int.): 302 [M]⁺ (10), 165 [C₉H₉O₃]⁺ (100).

4.6. Biological evaluation

The in vitro sensitivity of *L. donovani* (MHOM/ET/67/L82) amastigotes to test compounds was determined in a mouse peritoneal macrophage model (Neal and Croft, 1984). Mouse peritoneal macrophages were isolated from

CD 1 mice and maintained in RPMI 1640 medium with 10% heat-inactivated fetal calf serum and 50 µg/ml gentamycin at a density of 10⁵ cells ml⁻¹ in eight well tissue-culture chamber slides (Labtek Products, Miles Laboratories) at 37°C in a 5% CO₂/air mixture. After 24 h, the macrophages were infected with *L. donovani* amastigotes isolated from the spleen of an infected hamster, at a ratio of 10:1.

Test compounds were added to the infected macrophages in fresh medium 24 h later, on day 2, and again on days 4 and 7. Each compound was tested in quadruplicate, initially at 300, 100, 30 and 10 µM. On day nine, the macrophage monolayers were fixed with methanol and stained with Giemsa stain. The percentage of infected cells was determined. Damage to macrophages was assessed in order to determine toxicity. Where selective anti-leishmanial activity was found, the compound was re-tested at an appropriate range of concentrations. ED₅₀ values were calculated by linear regression analysis with P₉₅ fiducial limits.



4.7. *In vitro* promastigote assays

Assays were carried out in 96-well microplates using *L. donovani* promastigotes grown in Schneider's medium with 10% fetal calf serum. Each compound was tested in quadruplicate at 100, 30 and 10 μ M with a promastigote suspension at 10^6 /ml per well, and incubated at 24°C for 48 h. Promastigote motility was assessed by inverted microscopy.

4.8. *In vivo* testing against *Leishmania donovani*

Female BALB/c mice (B and K Ltd.), 18–20 g, were infected by the tail vein with 5×10^6 amastigotes of *L. donovani* derived from a hamster spleen. One week after infection, mice were individually dosed subcutaneously, once per day for five days, with compounds **7**, **24**, **25**, **26** and **27** suspended in 0.25% cellacol. Three days after completion of drug treatment, mice were sacrificed, livers were removed, weighed, and smears prepared. The liver amastigote load of untreated and treated mice was calculated from liver weight (mg) $\times$ number of amastigotes per 500 liver cells.

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